

"Ferripolyphosphate" as a Whey Protein Precipitant

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"Ferripolyphosphate," a liquid complex of ferric ion with a long-chain polyphosphate, precipitates virtually all the protein in commercial acid whey at pH 3.2–4.0. A typical precipitate contains 22% protein, 12% iron, 39% P_2O_5 , and 5% calcium.

The lyophilized product is white, fluffy, and has a mildly acidic flavor. This precipitation technique is of potential value for recovering high quality protein from industrial discharges of whey in a form useful for enrichment of foods with iron.

The precipitation of whey proteins at acid pH by the polyphosphate anion (Gordon, 1945) and by heavy metal cations, including Fe(III) (Block and Bolling, 1955) has been described. This paper describes the preparation of an iron polyphosphate-protein powder from cheese whey by means of a precipitation technique using "ferripolyphosphate" (Hazel *et al.*, 1968), a soluble complex of ferric ions with a long-chain polyphosphate. The powders, containing up to 15% iron, are white, fluffy, mild tasting, and uniform in texture. A preliminary report of these has been made (Jones *et al.*, 1971).

EXPERIMENTAL

Preparation of "Ferripolyphosphate." Soluble "ferripolyphosphate" complex was prepared from ferric chloride and the sodium polyphosphate glass, Calgon (Calgon Corporation, Pittsburgh, Pa.). Ferric chloride solution, 0.5 *M*, was freshly made at room temperature from reagent grade solid iron(III) chloride hexahydrate and distilled water. A solution which was 3 *M* in sodium phosphate monomer was prepared at room temperature by dissolving 30.6 g of unadjusted powdered Calgon in distilled water to give 100 ml of solution. Calgon was easily dissolved in a Waring Blender. Potentiometric titrations were used to determine average phosphate chain lengths (Van Wazer *et al.*, 1954) in solutions prepared both with and without the blender, and no difference in chain length was found. The phosphate and iron solutions were cooled to 10°C and mixed quickly in a blender. Subsequent experiments indicated that neither the cooling of the solutions nor the order of mixing was essential to prepare satisfactory soluble complexes.

Three forms of "ferripolyphosphate" were prepared which differed from each other in their relative proportions of iron and phosphate. These forms were a sparingly soluble solid gel, a liquid system 0.25 *M* in iron, and a liquid system 0.167 *M* in iron. Combining one volume 0.5 *M* ferric chloride with one-half volume 3 *M* Calgon produced a gel with an Fe/P mole ratio of approximately 1/3. Although poorly soluble in water, the gel dissolved readily in excess sodium polyphosphate solution. Thus, a liquid system having a pH of 1.2 and an Fe/P mole ratio of approximately 1/6 was produced by mixing equal volumes of ferric chloride and Calgon. The liquid complex system, 0.25 *M* iron, was clear and light orange in color. After standing at 25°C for about 3 days, or at 4°C for about a week, a large amount of white precipitate accumulated in the container. The liquid remaining was pink in color and was an effective protein precipitant.

Mixing one volume of 0.5 *M* ferric chloride with two volumes of 3 *M* Calgon produced a liquid system having a pH of 2.2 and an Fe/P mole ratio of approximately 1/12 (0.167 *M* in Fe). After standing at room temperature for about 4 days, the initial pale orange color changed to pink. Some preparations contained suspended solid material which settled in 24 hr. In these same preparations, precipitation within the system commenced in about 10 days at room temperature. Precipitation could be retarded by storage at 4°C. Some other 1/12 preparations contained no suspended solid and remained clear indefinitely at room temperature. Such variations in stability were apparently related to differences in quality of the reagent grade ferric chloride from different suppliers. In the experiments described here, the "ferripolyphosphate" soluble complex of mole ratio 1/12 was used extensively because of its demonstrated stability, as compared to the 1/6 complex, at low temperature over a period of months. Polyphosphate complexes of ferrous chloride were prepared, but did not cause precipitation of protein. Ferric nitrate complexes behaved like ferric chloride complexes.

Precipitation of Whey Proteins. Sufficient "ferripolyphosphate" was added at room temperature to whey at pH 4.6 to bring the concentration of iron to a desired level. Routinely, 250 ml of mole ratio 1/12 "ferripolyphosphate" was added for each liter of whey to give an iron concentration of 0.041 *M* or 2.29 g/l. A white floc was generated with the addition of even small amounts of "ferripolyphosphate." The pH of the system normally fell into the range 3.5–3.8, or it was adjusted to that pH. Precise control of pH within the range 3.2–4.0 was not necessary to obtain satisfactory powders. In 4 to 6 hr the precipitate had settled sufficiently so that the supernatant liquid could be siphoned from the vessel. The remaining liquid was removed by centrifugation. The precipitate was washed with distilled water, dialyzed exhaustively against distilled water at 4°C, and lyophilized.

All experiments noted here were conducted with acid whey obtained as a cottage cheese by-product from Sealtest Dairies, Chambersburg, Pa. The whey as obtained contained relatively large amounts of calcium ions and a low protein level which varied between 0.2 and 0.3% (w/v). (Acid whey prepared in the laboratory by acidifying raw skim milk to pH 4.6 and removing the precipitated casein contained about 0.8% protein.)

Analytical Methods. Iron analyses were performed by atomic absorption spectrophotometry. Phosphorus analyses were by the method of Meun and Smith (1968). Nitrogen determinations were by the Kjeldahl method, using a conversion factor of 6.25. Zone electrophoresis of some "ferripolyphosphate"-protein powders (hereafter referred to as FIP-protein powders) was performed in 8% polyacrylamide gel in pH 9.2 TRIS (trihydroxymethylaminomethane) buffer,

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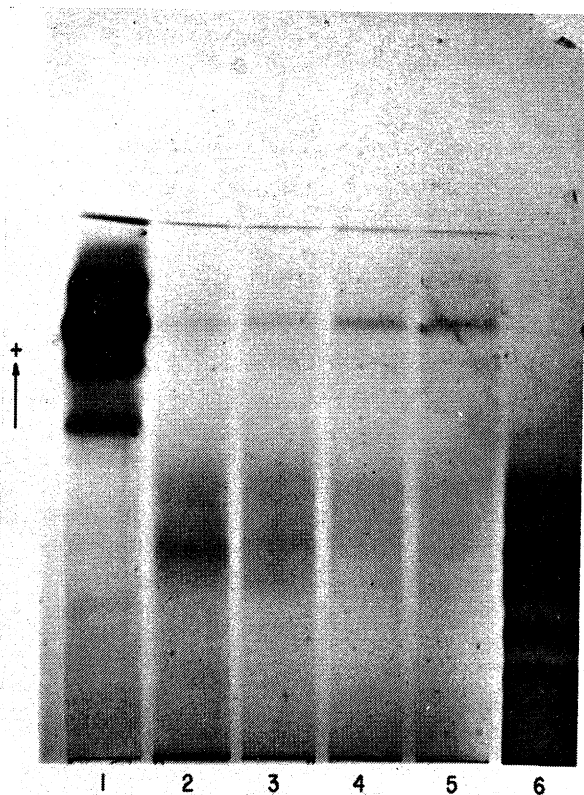


Figure 2. Polyacrylamide gel electrophoresis in 4.5 M urea of FIP-protein powders prepared by adding 3, 5, 10, and 20-ml volumes FIP to 100-ml portions of whey (slots 2-5). Standards are α -lactalbumin (slot 1) and β -lactoglobulin (slot 6)

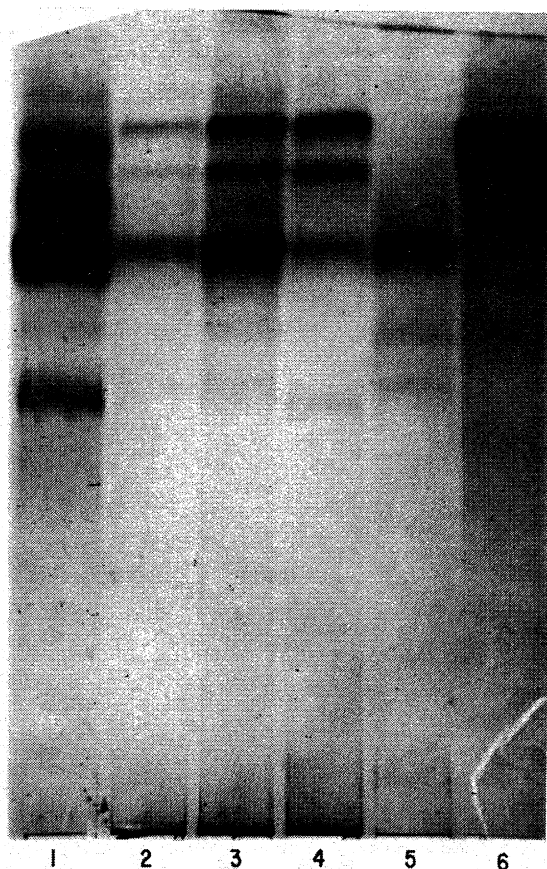


Figure 3. Polyacrylamide gel electrophoresis, no urea, of FIP-protein powders (slots 2-5), α -lactalbumin (slot 1), and β -lactoglobulin (slot 6). See text for details

Table IV. Disposition of Protein in FIP-Protein Powder and Remaining Supernatant

	FIP-protein powder	Supernatant ^a
Material yield, g	70 ^b	21.0 ^c
Protein, %	22.2	30.2
Protein, g	15.6	6.34
Protein expected, g ^d	21.2	
Protein precipitated, %	74	
Protein left in supernatant, %		30

^a Liquid remaining after precipitate was removed. ^b From 7300 ml of whey + 1800 ml of 1/12 "ferripolyphosphate." ^c Based on a portion of the supernatant which was dialyzed and lyophilized. ^d The protein content of this commercial whey was 290 mg/100 ml.

the multiple banding customary in urea gels. The four FIP-protein powders in slots 2-5, respectively, represent additions of 3, 5, 10, and 20 ml of "ferripolyphosphate" solution to 100 ml of commercial whey. The patterns indicate that FIP at low concentrations causes precipitation mainly of β -lactoglobulin, and that at higher levels of FIP, the precipitate contains greater amounts of α -lactalbumin.

Figure 3 is a non-urea gel containing α -lactalbumin and β -lactoglobulin in slots 1 and 6, respectively. Slots 2 and 3 contain powders prepared with "ferripolyphosphate" solution 8 weeks old and 24 hr old, respectively. Table II contains a further description of these powders. It should be noted that the FIP-protein powder obtained with the aged "ferripolyphosphate" contains more total protein (119 mg), whereas the powder obtained with the freshly prepared "ferripolyphos-

phate" (24 hr old) contains more protein per unit weight (about 0.45 mg/mg vs. 0.20 mg/mg). This difference apparently accounts for the heavier staining observed in slot 3.

Slot 4 is another FIP-protein powder prepared by the addition of 250 ml of "ferripolyphosphate" (mole ratio 1/12) per liter of commercial whey. As part of the processing of this powder, the supernatant liquid was dialyzed and lyophilized. A sample of the solids recovered in this manner is in slot 5. It is apparent that under these conditions, β -lactoglobulin and α -lactalbumin were precipitated, but that some α -lactalbumin remained in soluble form in the supernatant. Table IV gives the compositions of the powder and supernatant residues in slots 4 and 5.

"Ferripolyphosphate" appears to be a useful reagent for the recovery of highly nutritious proteins from whey. The proteins can be obtained in a form containing 12-15% iron, which is an upgrading of the nutritional value of the proteins. Thus, they could serve as important iron-enriching additives to dairy products and other commonly used foods; *e.g.*, flour, cereals, and potato flakes. This is of particular importance in light of recent nutritional surveys (ARS 62-18, 1969) indicating insufficient iron in the diets of some segments of the population.

ACKNOWLEDGMENT

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LITERATURE CITED

Block, R. J., Bolling, D., to the Borden Co., U.S. Patent 2,710,858 (June 14, 1955).